Two-Dimensional Chemotherapy Simulations Demonstrate Fundamental Transport and Tumor Response Limitations Involving Nanoparticles

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ABSTRACT

We present multiscale computer simulations of the delivery of chemotherapy and the tumor cells' response to the therapy. Even in a best-case scenario of: constant drug release from the nanoparticles; one cell type, which is drug-sensitive and does not develop resistance; targeted nanoparticle delivery; and for model parameters calibrated to ensure sufficient drug or nanoparticle blood concentration to rapidly kill all cells *in vitro*; our analysis shows that convective and diffusive transport limitations *in vivo* are severe and that drug levels inside the tumor are far less than *in vitro*, leaving large parts of the tumor with inadequate drug concentration. The *in vivo* rate of tumor shrinkage is several orders of magnitude less than *in vitro*, and after some shrinkage the tumor may achieve a new mass equilibrium far above detectable levels. Adjuvant anti-angiogenic therapy "normalizing" the vasculature may ameliorate transport limitations, although leading to unwanted tumor fragmentation.

Keywords: chemotherapy, nanoparticles, computer simulation, tumor fragmentation

1 INTRODUCTION

Drug delivery through nanoparticles presents significant potential advantages over traditional delivery *via* bolus injection. Taking advantage of the pore size differential between tumor and normal vasculature, long-circulating nanoparticles could be sized so as to extravasate only from tumor vasculature [1,2]. Such targeted delivery could greatly reduce drug tissue toxicity and make it possible to choose moderate, constant doses leading to long time periods of cell exposure to the drug over pulsed maximum tolerated dosing. For nanoparticles on the order of 100 nm, the extravasated particles, being too bulky to diffuse, would release their drug via desorption of surface-bound drug, diffusion through the polymer wall, and nanoparticle erosion. In this way they would function as stationary sources of drug within the tumor. In vitro release experiments have shown that a near constant release can be maintained for months [3]. Smaller particles capable of diffusion throughout the tumor interstitium could also be employed: 1—10 nm particles have been demonstrated capable of targeting cancer cells [4]. Combining the strategies of tumor-specific extravasation and cell-specific constant dose targeting ("smart" delivery) has the potential to greatly increase the efficacy of the drug while simultaneously reducing host tissue toxicity.

However nanoparticle chemotherapy strategies (and traditional chemotherapy) face delivery limitations due to poor transport. The tumor vasculature is notorious for its irregularity [5,6,7]. The hyperpermeable vasculature [8] together with a lack of a functional lymphatic system results in increased fluid pressure within the tumor [7]. In addition to interstitial fluid pressure, a tumor has a separate mechanical pressure associated with cellular proliferation [9] that plays a key role in the collapse of tumor vessels and further restriction of the blood supply. The blood flow in tumors grown in transparent windows has been investigated and found to be intermittent, periodically abating and reversing [6,10]. The extravasation of a macromolecule or a nanoparticle from a blood vessel depends primarily upon convection. As a result of the abnormal features of tumor vasculature, the convective transport of molecules and particles is compromised. Once a molecule or a particle has extravasated from a blood vessel, it must diffuse across the interstitium. The time required for a molecule to diffuse across tumoral tissue may be of the order of days for a 1 mm distance, and months for a 1 cm distance [6,7]. In a hypothetical tumor uniformly perfused with vessels about 200 μm apart, with sufficiently high interstitial pressure to stop fluid extravasation in the center, and with some collapsed vessels due to cell proliferation, it may take from days to months for a macromolecule to diffuse into the center [6]. Thus, considering only convection from the blood vessels and diffusion across the tumor interstitium, a molecule faces impediments to its final delivery. It has been proposed that tumor vasculature could be "normalized" via antiangiogenic therapy [11]. Pruning immature and inefficient blood vessels may lead to a more normal vasculature of vessels reduced in diameter, density, and permeability, which may in turn lead to lower interstitial pressure or cell-cell mechanical pressure [9,11].

In this article, we investigate these issues using multidimensional computer simulations [12]. The tumor simulator is based on a reaction-diffusion model of tumor progression [13] and angiogenesis [14] solved using a novel finite-element/level-set method [15] in two spatial dimensions coupled to an unstructured adaptive mesh technology [16] that allows efficient and accurate solution of the model equations. This implementation allows for the

first time the simulation of tumoral lesions through the stages of diffusion-limited dormancy, localized necrosis, vascularization and rapid growth, and tissue invasion in multiple spatial dimensions. We have incorporated [12] a simple model of nanoparticle delivery by convection from the bloodstream, and the release, diffusion and action of the anticancer drug contained therein. Very small, diffusing nanoparticles are also considered. The coupling of this chemotherapy model to the tumor simulator was previously developed [12]. Please refer to Ref. [12] for a complete description of the method and simulation studies. This enables us to directly simulate and quantify *in silico* the spatial dependence of tumoral tissue regression in the presence of anticancer drug on the heterogeneity of the tumor and vessel morphology, and on the internal mechanical pressure and drug concentration gradients, and thus to explore the benefits and limitations of nanoparticle chemotherapy.

2 SIMULATIONS OF NANOPARTICLE CHEMOTHERAPY

We present simulations of chemotherapy under the assumptions described previously [12]. We consider two ends of a spectrum—model one which best describes very small nanoparticles (or drug molecules) that are assumed to convect from the bloodstream and diffuse through the tumoral tissue; and model two that applies to large nanoparticles that are assumed to remain at their point of extravasation from the vasculature and function as a constant source of drug along the vessels. The drug itself diffuses through the tumoral tissue. This latter simulation is constructed to highlight difficulties in diffusion alone. We show that transport limitations impede the efficacy of the drug in causing tumor regression. We also indicate the possible benefits of improving drug delivery due to increased nanoparticle extravasation *via* the use of adjuvant anti-angiogenic drugs to "normalize" tumor vasculature [11].

The simulation shown in Fig. 1 (model one) illustrates features of convection/extravasation and diffusion of a number of different delivery carriers, such as free drug molecules, liposomes, current nanoparticles (only convection/extravasation), and nanoparticles in development, which are small enough to diffuse after they are extravasated. Therefore we consider nanoparticles at a constant blood serum concentration being convected continuously through the blood vessels, extravasating and diffusing (see Ref. [12] for details).

An inspection of the morphology of the tumoral tissue reveals a non-uniform distribution of newly formed blood vessels around which tumor cells are proliferating (thick boundary). Scattered islands of necrotic cells are also visible (thin boundaries). In addition the pressure distribution due to cellular proliferation is highly non-uniform. It is clear that the local level of chemotherapy agent after extravasation (and the tumor response) will be the result of the competition of pressure opposing convection and density of vessels that favors delivery. The delivery is indeed highly non-uniform. In particular, it is immediately apparent that the highest delivery of chemotherapy agent occurs in the central region of the 2-D lesion. This is a result of the competition of a high pressure and a high density of vessels observed. We have run a number of simulations under similar conditions [12] that reveal that the outcome of this competition is not always in favor of increased extravasation. As a consequence of the non-uniform delivery of the chemotherapy agent, Fig. 1 reveals that tumor regression is likewise non-uniform, being maximum around areas of maximum drug extravasation. Note also that tumor cell regression causes a local decrease of pressure, which, in turn, causes a local increase in drug delivery [9]. After the chemotherapy agent is extravasated, penetration into the tumoral tissue is the result of the competition of diffusion and uptake by the cells. This strongly limits the area around the vessels that is permeated by the diffusing chemotherapy agent.

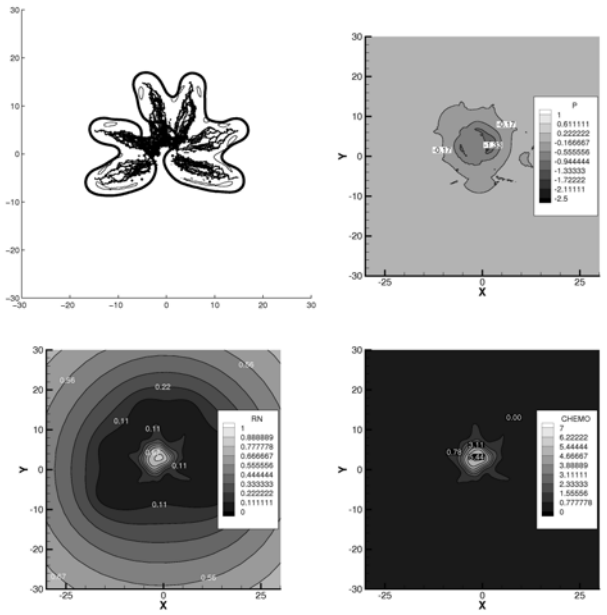

Figure 1: Chemotherapy simulation (model one). "P" is pressure, "RN" nutrient concentration, "CHEMO" drug carrier level. Lesion has achieved a stable equilibrium of cell proliferation and death

In Fig. 2, the dashed line labeled 'B' represents the tumor mass regression versus time. By t = 1200 the tumor undergoing chemotherapy has regressed but has stabilized at a dimensionless mass of about 471, or 89% of its value before therapy [12]. By examining the levels of chemotherapy agent (drug molecules or nanoparticles) extravasated and diffused into the tumor in Fig. 1 we see that they are far below that in the blood serum. This means that in a large part of the tumor the net growth rate is still positive. As an indication of how significantly the action of

the drug is compromised by poor transport, we computed [12] the fastest rate of regression from curve "B" in Fig. 2 and obtained a value of almost four orders of magnitude less than the *in vitro*! Even though levels of anticancer agent in the tumoral tissue are now approaching those in the blood serum, the tumor has split into two parts, leaving a cleft [9] right where most of the drug is.

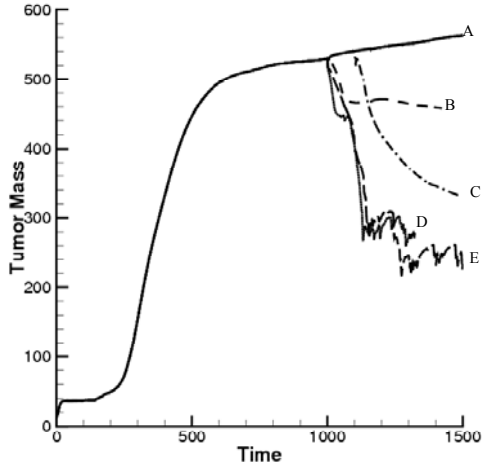

Figure 2. Tumor mass vs. time. A: Tumor growth without chemotherapy; B: Chemotherapy simulation involving small, diffusing chemotherapy carriers (model one); C: Chemotherapy simulation involving large, non diffusing nanoparticles but in conjunction with adjuvant anti-angiogenic therapy (model two); D, E: Simulations corresponding to B and C but assuming higher blood vessel mobility.

The previous simulation has shown that because of non-uniform extravasation and diffusion, the distribution of nanoparticles in the tumoral lesion and the cells' response and regression will also be non-uniform. Anti-angiogenic therapy has been proposed to normalize tumor vasculature and overcome convection limitations by pruning and regularizing the vasculature and thus either lowering interstitial fluid pressure or the cell-cell mechanical pressure due to cell proliferation [7,9]. In the first case, the lowering of interstitial pressure will increase extravasation from the blood vessels. In the second case, the lowering of mechanical stress may return collapsed tumor vessels to a functional state thus again leading to increased extravasation. To predict the best possible outcome of adjuvant anti-angiogenic therapy, we assume in the following simulation (model two) that a uniform and adequate concentration of nanoparticles has been effected at all perfused regions of the lesion independent of local pressure, and that they sit at the extravasation site releasing drug at a constant rate [12]. This describes large, 100 nm particles that don't diffuse once extravasated. Instead, the drug molecules are released from the nanoparticles and diffuse into the tumoral tissue. Thus here we isolate the effect of diffusional limitations of drug after release from the nanoparticles.

The dimensionless tumor cells' mass versus time is given by the dotted-dashed line labeled 'C' in Fig. 2, with therapy beginning in this case at time $t = 1100$. Note that in this simulation (model two) pressure is assumed to be irrelevant to extravasation. Fig. 3 reveals that because of the more uniform source of drug along the vessels the number of tumor cells reached by the drug is more extensive, resulting in greater tumor mass regression. However, because of heterogeneous distribution of blood vessels, and of diffusion/uptake of the drug by the tissue, the drug distribution illustrated in is still far from uniform, once again leading to the stabilization of tumor mass after regression at about 65% of the mass before treatment. It is important to note that the rate of tumor shrinkage is still three orders of magnitude less than the rate predicted *in vitro* [12].

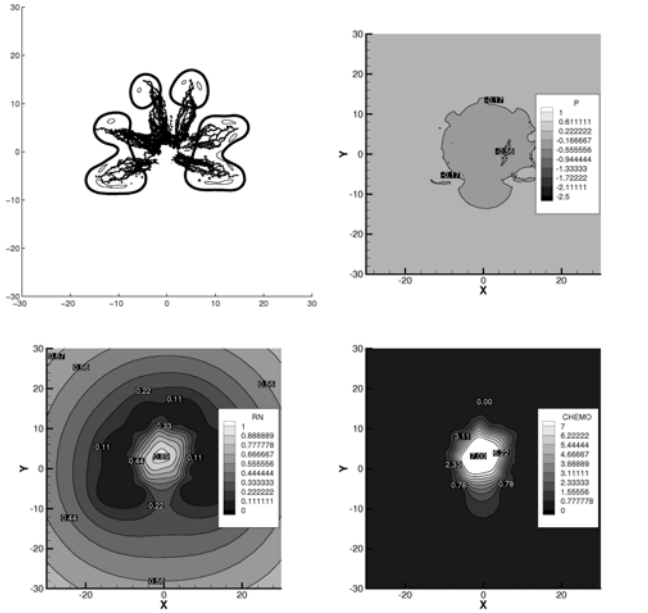

Figure 3. Chemotherapy simulation (model two), involving large nanoparticles and adjuvant anti-angiogenic therapy, at time $t = 1400$ when the tumor mass has stabilized after some regression. Note tumor fragmentation induced by the therapy.

The tumoral lesion morphology illustrated in Fig. 3 as predicted by our simulation reveals that tumor cell regression has lead to mass fragmentation in separate clusters of surviving tumor cells. This observation is in agreement with recent experimental results following anti-angiogenic therapy [17,18,19]. The underlying mechanism is nonuniform cell proliferation and death. Anti-angiogenic therapy increases local levels of drug and thus of cell kill, thus leading, in the presence of weak adhesive forces, to diffusional instability characterized by separation of clusters of tumor cells that concentrate where the nutrient and drug levels are optimal for survival.

3 CONCLUSIONS

We have quantitatively and graphically demonstrated the impact of fundamental transport limitations to the delivery of anticancer drug through a range of delivery modes, including nanoparticles, and to the resulting tumor response and regression [12]. We have assumed a best-case scenario involving a single cell clone, which is drug-sensitive and does not develop resistance, no host tissue toxicity, and no specific or non-specific binding or metabolism of drug molecule carrier within the tumor. Importantly, the convective and diffusive transport limitations demonstrated apply not only to current technologies and modes of delivery, but also to very small nanoparticles currently in development capable of diffusing through the tumor interstitium and selectively targeting cancer cells.

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